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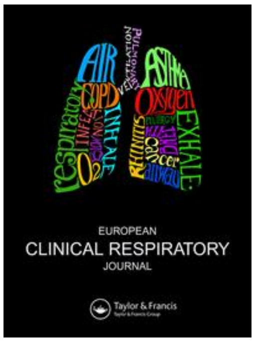

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Household dampness and microbial exposure related to allergy and respiratory health in Danish adults

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ABSTRACT

Background: Indoor dampness has consistently been associated with respiratory symptoms and exacerbations. The causal mechanisms may involve increased microbial exposures. However, the evidence regarding the influence of indoor microbial exposures under damp- and non-damp conditions on the risk of asthma and allergy has been inconclusive.

Objective: The aim of this study was to investigate the association between dampness and microbial exposure with allergy and respiratory health in Danish adults using a cross-sectional design.

Methods: From 1,866 participants of the Health2006 cohort, we selected three non-overlapping groups: 196 at random, 107 with confirmed atopy, and 99 without atopy. Bedroom dust was sampled using electrostatic dust fall collectors and analysed for endotoxin, β-(1,3)-D-glucan, 19 microbial species or groups, and total fungal load. Household moisture-related problems and asthma were self-reported by questionnaire. Atopy was determined by skin-prick-testing and lung function was measured by spirometry.

Results: Household moisture damage was positively associated with asthma outcomes, although this was statistically significant only in atopics for self-reported asthma (odds ratio (OR) 3.52; 95% CI 1.01–12.7). Mould odor was positively associated with wheezing (OR 6.05; 95%CI 1.19–30.7) in atopics. Inconsistent associations were found for individual microbial exposures and health outcomes. Inverse associations were observed between microbial diversity and rhinitis in the random sample and both doctor-diagnosed and self-reported asthma in non-atopics.

Conclusions: In conclusion, our findings suggest that household moisture damage may increase the risk of asthma and wheeze with mould odor in atopics. In addition, asthma and allergy may be affected by the indoor microbial composition in urban domestic environments. Further studies are needed to identify and understand the causal agents and underlying mechanisms behind the potential effects of environmental microbial exposure on human health.

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Moisture and mould; household microbial exposure; endotoxin; house dust; respiratory health; allergy; asthma

Introduction

The prevalence of household dampness in European dwellings has been estimated to be around 15% [1] and reports for Danish residences have estimated the prevalence of self-reported dampness problems to be: 11% (1995); 8% (1997); 9% (1999); 11% (2000) [2]. Indoor dampness has consistently been associated with respiratory symptoms and exacerbations, and there is some evidence for association with new-onset asthma and allergies [3–7] and a stronger negative health effect of household dampness in atopics [1]. The causal mechanisms may involve increased microbial exposures, but the evidence is inconclusive.

To date, most epidemiological studies have been limited to assessing health effects associated with exposure to microbial components, especially endotoxin, and culturable moulds [8]. The results have been inconsistent, suggesting protective, detrimental, and no health effects in relation to asthma and allergy [8]. However, comparisons between studies have been hampered by the lack of standardization and validation of methods for measuring indoor microbial exposure. Recently, the use of molecular techniques such as quantitative polymerase chain reaction

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Supplemental data for this article can be accessed here

(qPCR) to identify specific viable and non-viable bacterial and fungal agents to assess indoor exposure has become more common. Although some associations between single microbial markers and asthma outcomes have been demonstrated, there are only a few studies using qPCR and these have largely focused on respiratory health effects in children [9–13]. Nonetheless, due to the high sensitivity and reproducibility of qPCR, this method has been recommended for epidemiological investigations to assess the role of individual species or genera in the development and expression in respiratory and allergic diseases [14–17].

Besides focusing on individual microorganisms, there is also an interest in the potential effect of quantity and diversity of microbial exposure on respiratory health. Greater diversity of fungal and bacterial agents has been shown to reduce the risk of asthma in children living on farms [18]. Similarly, one study found an inverse association between total fungal DNA and wheeze and nighttime cough in Spanish school children [19]. In contrast, another study found a positive association between elevated levels of total viable mould and risk of rhinitis and night cough and persistent cough [12], whereas others found no associations between total fungal DNA and respiratory health outcomes [11]. These studies highlight the need to study both the potential effect of individual microbial exposures and quantity and diversity of these exposures. Therefore, the aim of our study was to evaluate the prevalence of dampness and microbial exposure in urban homes and assess their association with allergy and respiratory health in adult residents. We also assessed whether health effects associated with dampness and microbial exposure were different for atopics and non-atopics as atopics may be more susceptible.

Material and methods

Study design and study population

This was a cross-sectional study nested within the Health2006 cohort. The baseline Health2006 cohort was randomly drawn from the urban background population aged 18–69 years, living in 11 municipalities in the south-west of suburban Copenhagen [20]. A total of 3,471 individuals (45%) entered the study and underwent health examination at the Research Centre for Prevention and Health (Glostrup University Hospital, Copenhagen) between 2006 and 2008. In 2011–2012, the baseline Health2006 cohort was invited for a 5-year follow-up examination following essentially the same study protocol [21]. A total of 3,405 were eligible for a follow-up invitation and of these, 2,308 (69%) were reexamined between November 2011 and November 2012. Of those, 1,866 participants completed questionnaires on their home indoor environment and consented to collection of settled dust from their bedroom. For this study, we selected three distinct groups. We extracted a random sample of 196 participants reflecting the 5-year follow-up population of the Health2006 cohort. For investigation of potential different reactions related to atopic status, we added two groups in the age-range 30 to 50 years; a random group of 107 participants with atopy (described below) and a group of 99 non-atopic participants. The design and selection of participants is illustrated in Figure 1. The study was approved by the Ethical Committee of the Capital Region of Denmark (H-3-2011-080) and by the Danish Data Protection Agency (2006-53-1283). Participants had received written, oral and video information about the project and provided written consent prior to participation.

Exposure assessment

Household dampness and other indoor environment characteristics

Dampness was determined based on self-reports to yes/no-questions of four dampness indicators: (1) *Have you noticed any damp stains on surfaces, i.e. floor, walls or ceiling in your bedroom?* (damp stains in bedroom); (2) *Is there any odor of moisture or mould in your home?* (mould odor); (3) *Is there any condensation on the inside of your windows?* (condensation on windows); and (4) *Have you ever had moisture damage in your home?* (moisture damage). Additionally, participants reported the following information: hours spend in the home during one weekday, type of dwelling (apartment, detached house, attached house, other type of dwelling), crowding (occupants per square meter), pet keeping (yes/no), and smoking (daily, occasionally, ex-smoker, never smoker).

Dust sampling, extraction and analysis

Detailed information on dust sampling, extraction and analysis is provided in the supplementary information. Briefly, settled house dust was collected by participants using an electrostatic dust fall collector (EDC) (Zeeman, Utrecht, the Netherlands) placed 1.5 m above the floor in the participant's bedroom during a 14-day period [22]. The EDC was returned in an envelope to Aarhus University and no later than 1 day after receipt, electrostatic cloths were stored at −20°C until undergoing extraction for dust and bio-aerosols in a sterile liquid solution as described by Noss et al. [22].

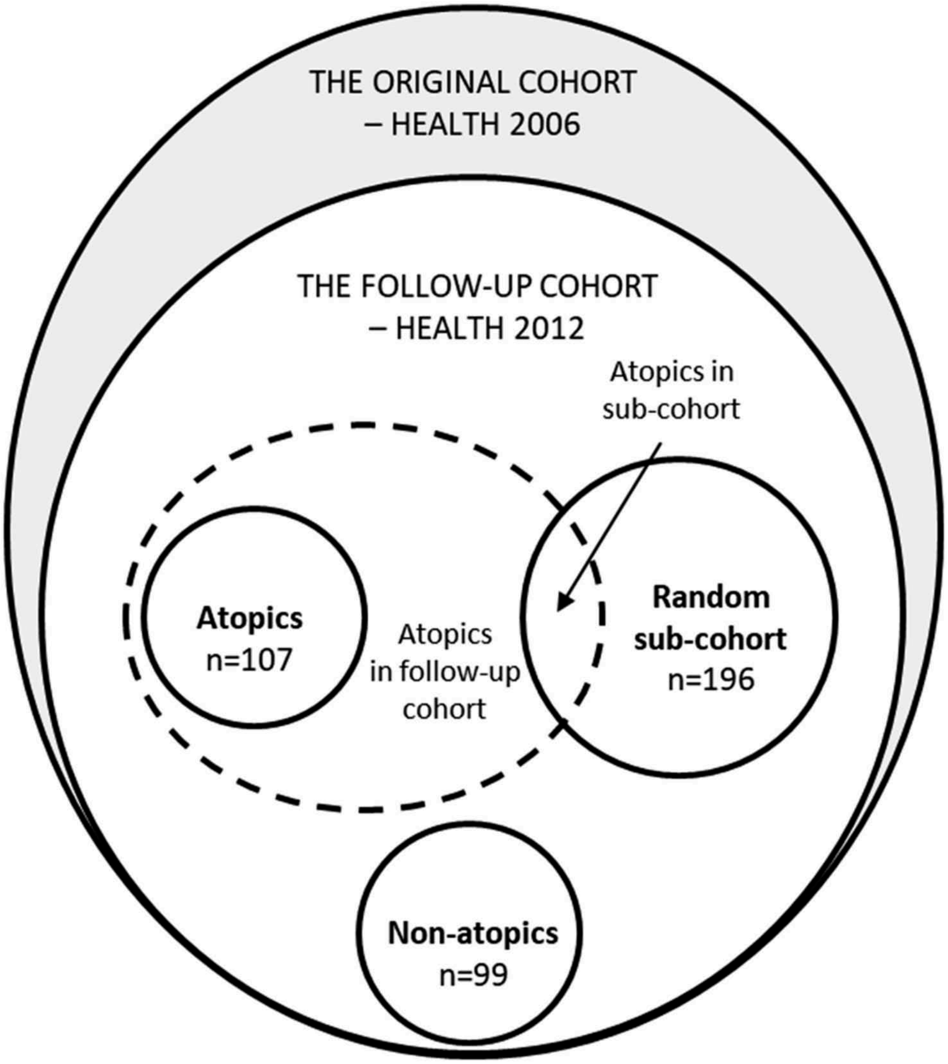

Figure 1. Study design and selection of participants.

Two microbial components, endotoxin and β-(1,3)-D-glucan, were quantified using the Limulus Amebocyte Lysate (LAL) assay. The detection limits (LOD) were 9.6 EU/m^2 for endotoxin and 366 μg/m^2 for β-(1,3)-D-glucan. Among samples, 13% were below detection limit for endotoxin and 10% for β-(1,3)-D-glucan. These samples were assigned half of the LOD value [23]. Data were presented as loads equivalent to endotoxin units (EU) per m^2 (EU/m^2) and β-(1,3)-D-glucan in μg per m^2 (μg/m^2).

qPCR assays were prepared using SYBR Green Master Mix (Bio-Rad Laboratories Inc., California, USA). DNA from the following microorganisms was detected using primers for (1) one group of bacteria (*Streptomyces spp.*), (2) 15 fungal species (*Acremonium strictum, Alternaria alternata, Aspergillus fumigatus, Aspergillus versicolor, Aspergillus niger, Chaetomium globosum, Cladosporium cladosporioides, Cladosporium herbarum, Cladosporium sphaerospermum, Penicillium chrysogenum, Rhizopus stolonifer, Stachybotrys chartarum/chlorohalonata, Trichoderma viride, Ulocladium chartarum, Wallemia sebi*), (3) three fungal groups (*Mucor/Rhizopus spp., Aspergillus glaucus spp., Penicillium/Aspergillus/-Paecilomyces variotiii spp.*), and (4) one universal fungal primer for the total fungal load. Standard curves were produced for respective analysis based on total genomic DNA extracts from pure cultures and were quantified using limiting dilution analysis [24]. Microorganisms were calculated at cell level, expressing results as cell equivalents (CE) per m^2 (CE/m^2) referring to the number of spores per m^2.

Outcome assessment

Atopy and allergy

Skin-prick-testing (SPT) to a standard panel of 10 aeroallergens was performed by a trained nurse using the Soluprick SQ system (ALK Abelló A/S, Hørsholm,

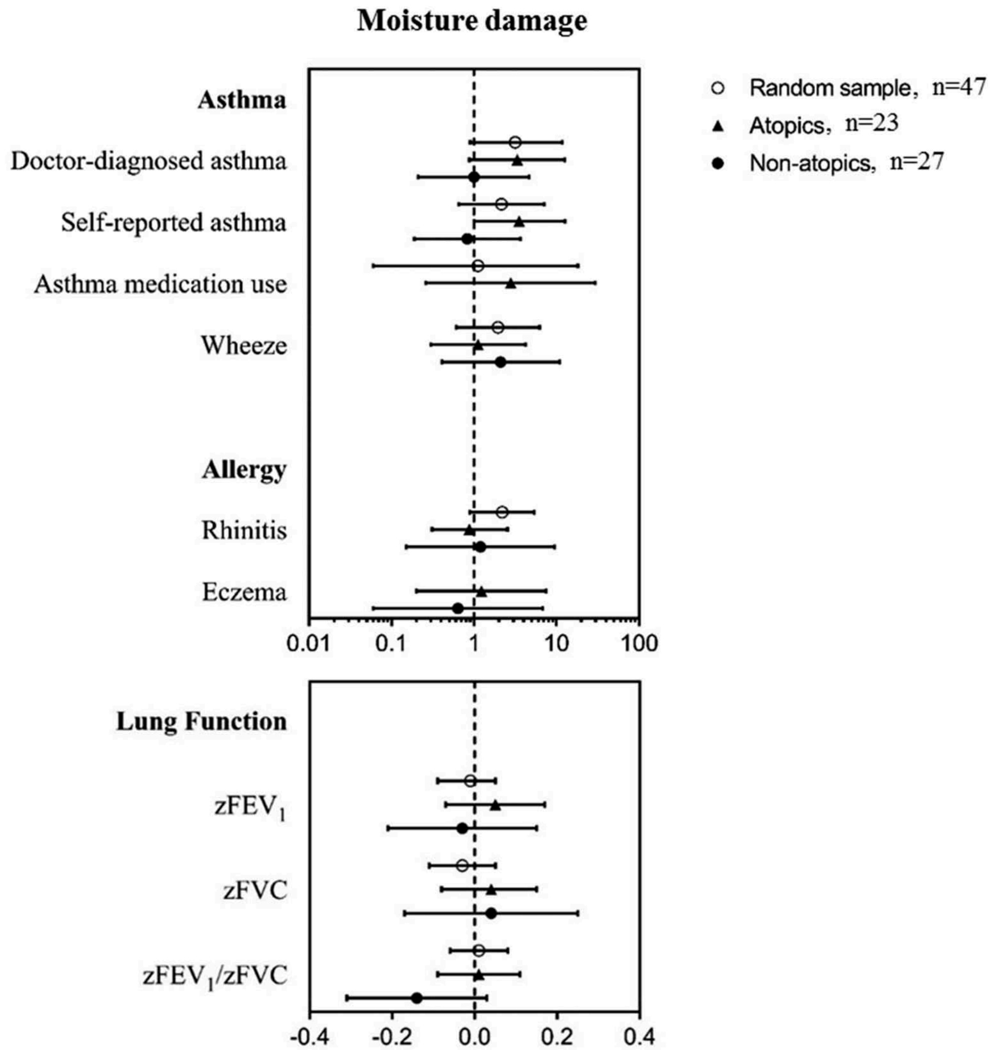

Figure 2. Associations of moisture damage with allergy, asthma and lung function in the random sample, atopics and non-atopics.

Denmark) and included the allergen extracts: (1) pollen: birch (*Betula verrucosa*), grass (*Phleum pratense*) and mugwort (*Artemisia vulgaris*); (2) pets: cat (*Felis domesticus*), dog (*Canis familiaris*), and horse (*Equus caballus*); (3) two house dust mites (*dermatophagoides pteronyssinus* and *Dermatophagoides farina)*; and (4) two moulds (*Cladosporium herbarum* and *Alternaria alternata)*. Histamine chloride was used as positive control and saline as negative control. Atopy was defined by having a positive test (mean wheal of 3 mm or more after subtraction of the negative control) to at least 1 of the 10 allergens. Participants were asked to abstain from use of antihistamines 3 days before the test. Allergic conditions (eczema and rhinitis) were identified by self-reported doctor diagnosis: *Has a doctor ever told you that you have eczema?* and *Has a doctor ever told you that you have rhinitis?*

Asthma

Asthma was determined by a positive response to each of the following four yes/no-questions: (1) *Has a doctor ever told you that you have asthma?* (doctor-diagnosed asthma); (2) *Have you ever had asthma?* (self-reported asthma); (3) *Do you currently use asthma medication?* (asthma medication use); and (4) *Have you had wheezing or whistling in your chest at any time?* (wheeze, the last 12 months) [25].

Lung function

Lung function was measured by spirometry according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [26] using Spiro USB Spirometers (MicroMedical Limited, Rochester, Kent, UK) [25]. Spirometers were checked daily with a 3-l calibrated syringe, and checked every 6 months with a decompression flow simulator [27]. Lung function data were obtained for 99% of the participants. Information on participant's sex, age, height, forced expiratory volume in first second (FEV_1) and forced volume capacity (FVC) were applied to calculate z-scores of FEV_1, FVC and the FEV_1/FVC-ratio based on the Global Lung Function Initiative (GLI) spirometry equations [28].

Statistical analyses

Initial analyses described basic-, indoor environmental-, and health characteristics. Chi-squared tests and t-tests were used to assess differences between atopic and non-atopic participants. Microbial loads (per m^2 of sampling area) followed a lognormal distribution and were presented as geometric means (GM) with geometric standard deviations (GSD). Differences in microbial exposure between bedrooms of atopics, non-atopics and the random sample were assessed by t-tests.

Multiple logistic regression analyses were conducted to assess associations between dampness indicators and microbial exposures and allergy and asthma outcomes. Multiple linear regression analyses were used to assess associations with lung function. In addition to specific microbial markers, we also measured associations with the total fungal load and microbial diversity calculated as the number of different detected fungi (excluding the fungal group of *Penicillium/Aspergillus/Paecilomyces varioti spp.*) and *Streptomyces* spp. (range, n = 1–18). All regression analyses were adjusted for sex, age, smoking, hours spent in the home, season, and additionally in sensitivity analyses for crowding (occupants per m^2), type of dwelling, indoor area (m^2), and pet keeping. Confounders were chosen *a priori* on the basis of previous evidence or *posteriori* due to differences in their association with selected groups. Multivariate results are presented as odds ratios (OR) for airway symptoms, doctor-diagnosed asthma, and allergy and as β-coefficients for lung function parameters, both with 95% confidence intervals (95% CI). The ORs and β-coefficients represent the estimated health effect expressed as the change associated with an increase in unit of load of microbial exposures, total fungal load, diversity or presence versus absence of dampness indicators. A significance level of $p < 0.05$ was applied. Statistical analyses were conducted using Stata version 13 (StataCorp LP, College Station, Texas, USA).

Results

Characteristics of the study population

Significant differences in characteristics between the study groups were found (Table 1). The atopic and non-atopic groups differed from the random sample on several characteristics: they were younger (due to the age-based selection), spent less time at home, had a larger living area, had more co-habitants, were more likely to keep pets, and were more likely to report condensation on windows. Generally, the atopic and non-atopic groups were similar in characteristics, but the atopic group was significantly more likely to report presence of condensation on windows and live in smaller homes. Sensitization against pollen allergens was most prevalent followed by pet- and house dust mite allergens (Table 2). Atopics were significantly more likely to report rhinitis, eczema, doctor-diagnosed asthma, self-reported asthma, and wheezing compared to non-atopics and to the random sample. Lung function was lower in atopic participants than in the random sample and non-atopic group, when taking into account age, sex, height and ethnicity shown in the z-scores.

Prevalence and diversity of microbial exposure

The most common microbes found in the dust were: *Aspergillus versicolor, Penicillium/Aspergillus/Paecilomyces varioti* spp., *Cladosporium* spp., *Streptomyces* spp., and *Wallemia sebi* (Table S1 (supplementary material)). Microbial loads varied greatly between homes, although at least three of the microorganisms were quantifiable in all samples (mean, 7; SD, 2.71). Significant seasonal variation was seen for several microorganisms. Cladosporium spp., Alternaria alternata and the total fungal load were highest during warm months (summer) and lowest during winter months, and Penicillium spp. and Aspergillus spp. was the opposite. Additionally, microbial diversity was lowest during winter (Table S2). On average, dust sampled from homes of atopics contained significantly less *Aspergillus fumigatus, Cladosporium sphaerospermum, Penicillium/Aspergillus/Paecilomyces varioti spp., Penicillium chrysogenum, Stachybotrys chartarum/chlorohalonata* than dust from homes of non-atopics (Table 3). Significantly less endotoxin and *Penicillium/Aspergillus/Paecilomyces varioti spp.* were found in dust samples from homes of atopics compared to the random sample. Significantly less *Acremonium strictum, Aspergillus fumigatus, Cladosporium herbarum, Cladosporium sphaerospermum, Penicillium chrysogenum, Stachybotrys chartarum/chlorohalonata*, but more endotoxin was found in dust from the households in the random sample, and the dust was less diverse in microbial composition than the dust sampled in the homes of non-atopics. Generally, positive but weak correlations were found between groups of microorganisms ($r < 0.50$), but subgroups of *Cladosporium* spp. were moderately to highly correlated ($r = 0.72$ to 0.88), as were *Cladosporium* spp. and total fungi ($r = 0.76$ to 0.83) (Table S3). Microbial diversity was significantly but only weakly to

Table 1. Characteristics of the study population.

	Random sample		Atopics		Non-atopics		
	n	(%)	n	(%)	n	(%)	p-value
Basic characteristics							
Number of participants	196	(100)	107	(100)	99	(100)	-
Sex							
Male	94	(48)	42	(40)	42	(42)	0.64[a]
Female	102	(52)	65	(60)	57	(58)	
Age (year), mean (SD)	54	(12.6)	42	(5.19)	44	(5.47)	0.21[b]
20-29 years	7	(4)	0	(0)	0	(0)	
30-39 years	18	(9)	32	(30)	26	(26)	
40-49 years	49	(25)	75	(70)	73	(74)	
50-59 years	43	(22)	0	(0)	0	(0)	
60-69 years	59	(30)	0	(0)	0	(0)	
>70 years	20	(10)	0	(0)	0	(0)	
Home characteristics							
Hours spend in the home, mean (SD)	14.6	(4.7)	12,8	(3.2)	13,1	(2.9)	0.59[b]
Type of dwelling							
Apartment	49	(25)	26	(24)	22	(22)	0.35[a]
Detached house	107	(55)	62	(58)	61	(62)	
Attached house/Rowhouse	39	(19)	18	(17)	15	(15)	
Other type of dwelling	1	(1)	1	(1)	1	(1)	
Indoor area (m^2)	126	(45.8)	122	(39.3)	137	(61.5)	**0.04[b]**
<70 m^2	15	(8)	10	(10)	6	(6)	0.68[a]
70-110 m^2	68	(35)	30	(28)	29	(30)	
>110 m^2	113	(57)	68	(62)	64	(64)	
Number of occupants	2,3	(1.01)	3,3	(1.25)	3,3	(1.18)	0.84[b]
1	31	(16)	12	(11)	10	(10)	0.40[a]
2	114	(58)	18	(18)	14	(14)	
3	20	(10)	16	(15)	24	(25)	
4	24	(12)	47	(44)	37	(38)	
≥5	7	(4)	14	(12)	13	(13)	
Crowding, mean (SD)	0.02	(0.01)	0,03	(0.01)	0,03	(0.01)	0.30[b]
Pet keeping (yes)	62	(32)	48	(45)	55	(56)	0.12[a]
Smoking, current (yes)	18	(9)	17	(16)	16	(16)	0.95[a]
Daily	13	(7)	12	(11)	10	(10)	0.30[a]
Occasionally	5	(3)	5	(5)	6	(6)	
Ex-smoker	51	(26)	32	(30)	41	(41)	
Never smoker	126	(64)	58	(55)	42	(42)	
Dampness indicators							
Damp stains in bedroom (yes)	12	(6)	12	(12)	8	(8)	0.45[a]
Mould odor (yes)	5	(3)	9	(8)	2	(2)	0.38[a]
Condensation on windows (yes)	48	(25)	43	(40)	34	(34)	**0.04[a]**
Moisture damage, ever (yes)	47	(27)	23	(25)	27	(29)	0.85[a]

Definition of abbreviations: n denotes number, SD denotes standard deviation. Statistical significance between atopics and non-atopics is denoted by a p-value <0.05.
[a]Chi^2-test.
[b]t-test.

moderately correlated with the individual microorganisms ($r = 0.18$ to 0.73). Few significant differences were found in microbial loads or indicators between homes with and without visible damp stains, mould odor, condensation on windows and moisture damage (Table S4). Among these, a higher load of endotoxin was detected for participant's reporting having mold odor indoors, whereas the load of endotoxin was lower among participant's reporting having damp stains in their bedroom.

Household dampness, allergy, asthma and lung function

Self-reported mould odor was positively associated with wheezing in atopics (OR 6.05; 95%CI 1.19 to 30.7) (Table S5). Additionally, moisture damage was associated with increased doctor-diagnosed asthma, self-reported asthma, and wheeze, although this was only statistical significant for self-reported asthma in atopics (OR 3.52; 95%CI 1.01 to 12.7) (Figure 2). When performing an overall analysis focusing on a potential interaction between dampness indicators and atopy on asthma outcomes, interaction was not found neither with mould odor (p = 0.44) nor with moisture damage (p = 0.15). No statistical significant associations were observed between dampness indicators and lung function and allergic diseases other than asthma, except for a lower zFVC in non-atopics reporting mould odor (β-coeff. −0.04; 95%CI −0.07 to −0.01).

Microbial exposure, allergy, asthma and lung function

Associations between microbial exposure and allergy, asthma and lung function are shown in supplementary

Table 2. Allergy, asthma and lung function in the random sample, atopics and non-atopics.

	Random sample		Atopics		Non-atopics		
	N = 196		N = 107		N = 99		
	n	(%)	n	(%)	n	(%)	p-value
Allergy							
SPT, any positive	64	(33)	107	(100)	0	(0)	**0.00[a]**
Pollen	49	(25)	84	(78)	0	(0)	**0.00[a]**
Pets	27	(14)	52	(48)	0	(0)	**0.00[a]**
Molds	4	(2)	10	(9)	0	(0)	**0.01[a]**
House dust mites	21	(11)	44	(41)	0	(0)	**0.00[a]**
Rhinitis	33	(17)	57	(53)	5	(5)	**0.00[a]**
Eczema	3	(2)	10	(9)	4	(4)	**0.01[a]**
Asthma							
Doctor-diagnosed asthma	14	(7)	21	(20)	10	(10)	**0.02[a]**
Self-reported asthma	16	(8)	22	(21)	11	(11)	**0.03[a]**
Asthma medication	8	(4)	9	(8)	4	(4)	0.92[a]
Wheeze	19	(10)	24	(22)	10	(10)	**0.01[a]**
Lung function							
Observed							
FEV_1 (l), mean (SD)	3.08	0.82	3.37	0.69	3.45	0.82	**0.01[b]**
FVC (l), mean (SD)	4.01	1.03	4.31	0.92	4.45	0.99	**0.03[b]**
FEV_1/FCV (%), mean (SD)	0.77	0.07	0.79	0.06	0.77	0.06	**0.01[b]**
z-scores							
$zFEV_1$, mean (SD)	−0.34	0.94	−0.49	0.75	−0.37	0.99	**0.03[b]**
zFVC, mean (SD)	−0.19	0.92	−0.28	0.75	−0.11	0.84	**0.05[b]**
$zFEV_1$/zFCV, mean (SD)	−0.31	0.95	−0.37	0.92	−0.48	0.97	0.81[b]

Definition of abbreviations: n, number; SD, standard deviation; SPT, skin prick test (atopic sensitisation); FEV_1, forced expiratory volume in first sec.; FVC, forced vital capacity; $zFEV_1$, z-score for FEV_1; zFVC, z-score for FVC; $zFEV_1$/FVC, z-score for the ratio between FEV_1 and FVC. Statistical significance between atopics and non-atopics is denoted by p-value <0.05.
[a]Chi^2-test.
[b]t-test.

information (Table S6-S8). For microbial components, higher loads of β-(1,3)-D-glucan and endotoxin tended to be associated with poorer health outcomes in terms of allergy, asthma and lung function, although only the association of endotoxin with self-reported asthma was statistically significant in the random sample (β-coeff. 1.71; 95%CI 1.05 to 2.78).

Analyses of individual species or groups of microorganisms showed generally inconsistent associations, and few of these associations were significant. In the random sample, inverse associations were statistically significant between *Streptomyces* spp., *Aspergillus versicolor, Cladosporium sphaerospermum* and rhinitis, and additionally between *Cladosporium herbarum* and eczema (Table S6). Furthermore, positive associations were shown for *Trichoderma viride* and rhinitis in both the random sample and in the non-atopic group, and also between *Chaetommium globosum* and rhinitis in the non-atopic group. In terms of respiratory outcomes, inverse associations were found for *Penicillium/Aspergillus/Paecilomyces varioti spp.* with doctor-diagnosed asthma and for *Aspergillus fumigatus* and *Cladosporium sphaerospermum* with self-reported asthma in non-atopics (Table S7). Moreover, positive associations were found between *Trichoderma viride* and self-reported asthma in non-atopics and between *Acremonium strictum* and wheezing in atopics. For lung function outcomes, a pattern of positive associations of species and groups of microorganisms with $zFEV_1$/FVC-ratio were shown in non-atopics, being statistical significant only for *Streptomyces* spp., *Aspergillus versicolor, Chaetommium globosum, Penicillium/Aspergillus/Paecilomyces varioti* spp. and *Wallemia sebi* (Table S8). In contrast, in the random sample inverse associations were found for *Alternaria alternata, Cladosporium cladosporioides, Cladosporium herbarum* with $zFEV_1$/FVC-ratio and for *Aspergillus niger* with zFVC, and in the atopics for *Chaetommium globosum* with $zFEV_1$.

Regarding microbial diversity and total fungal load, an overall pattern was observed with increasing diversity of microorganisms associated with a lower prevalence of rhinitis and asthma outcomes (Figure 3). This was, however, only significant among the random sample for rhinitis (OR 0.79; 95% CI 0.64 to 0.97) and in non-atopics for doctor-diagnosed asthma (OR 0.72; 95%CI 0.54 to 0.96), self-reported asthma (OR 0.78; 95%CI 0.61 to 0.98) and a higher $zFEV_1$/FVC-ratio (β-coeff. 0.09; 95%CI 0.03 to 0.16). Similarly, inverse associations were found for total fungi and eczema (OR 0.48; 95% CI 0.23 to 0.96) and $zFEV_1$/FVC-ratio (β-coeff. −0.12; 95%CI −0.24 to −0.01) in the random sample. Total fungi were associated with a higher $zFEV_1$/FVC-ratio (β-coeff. 0.17; 95%CI 0.01 to 0.32) in the non-atopics. When testing for interaction, we observed neither an increased nor decreased effect of diversity on the above-mentioned outcomes in relation to atopic status (results not shown). Furthermore, to increase power, we

Table 3. Microbial detection in household dust by the random sample, atopics and non-atopics.

	Random sample			Atopics			Non-atopics			Difference between atopics and non-atopics	Difference between atopics and the random sample	Difference between non-atopics and the random sample
	% >LOD[a]	GM	GSD	% >LOD[a]	GM	GSD	% >LOD[a]	GM	GSD	p-value[b]	p-value[b]	p-value[b]
Microbial components												
Endotoxin (10^3 EU/m^2)	92	0.21	5.29	64	0.04	9.16	91	0.05	3.70	0.61	**0.00**	**0.00**
β-(1,3)-D-glucan (µg/m^2)	92	3.55	3.37	87	3.87	4.02	-	-	-	-	0.62	-
Microorganisms (10^6 CE/m^2)												
Streptomyces spp.	82	2.45	3.17	82	2.58	3,13	78	2.98	4.52	0.43	0.73	0.21
Acremonium strictum	5	0.24	1.78	13	0.26	1.70	17	0.32	2.59	0.03	0.45	**<0.01**
Alternaria alternata	24	0.11	2.70	41	0.15	2.95	37	0.15	3.49	0.98	0.06	0.06
Aspergillus fumigatus	24	0.66	2.23	38	0.81	2.51	51	1.42	4.59	**<0.01**	0.10	**<0.01**
Aspergillus versicolor	94	3.54	3.75	97	3.13	3.19	93	3.81	4.08	0.30	0.45	0.66
Aspergillus niger	6	0.11	3.12	4	0.09	2.72	10	0.09	4.49	0.87	0.35	0.43
Aspergillus glaucus spp.	26	0.22	2.57	28	0.23	2.42	32	0.25	2.67	0.59	0.82	0.41
Chaetommium globosum	8	0.05	1.86	6	0.05	1.61	11	0.06	2.49	0.06	0.64	0.09
Cladosporium cladosporioides	95	9.38	4.31	99	13.4	3.75	90	10.4	7.03	0.26	0.07	0.59
Cladosporium herbarum	96	18.8	6.30	100	23.1	4.87	96	30.6	8.75	0.29	0.37	**0.04**
Cladosporium sphaerospermum	67	1.94	3.86	60	1.51	3.52	75	3.59	5.34	**<0.01**	0.15	**<0.01**
Mucor/Rhizopus spp.	15	0.56	2.29	12	0.52	1.71	17	0.58	2.20	0.30	0.44	0.66
Penicillium/Aspergillus/ Paecilomyces varioti spp.	99	37.3	3.84	99	27.0	3.31	100	40.0	3.61	**0.04**	**0.04**	0.67
Penicillium chrysogenum	14	0.17	1.81	15	0.18	1.79	27	0.23	2.31	**0.02**	0.75	**<0.01**
Rhizopus stolonifer	1	0.21	1.08	0	0.21	1.00	1	0.21	1.10	0.34	0.53	0.63
Stachybotrys chartarum/ chlorohalonata	5	0.05	1.37	2	0.04	1.39	7	0.06	2.72	**<0.01**	0.71	**<0.01**
Trichoderma viride	12	0.18	2.21	13	0.19	2.65	14	0.19	2.31	0.88	0.56	0.68
Ulocladium chartarum	6	0.05	1.46	7	0.05	1.75	9	0.05	1.60	0.94	0.31	0.28
Wallemia sebi	42	0.94	3.07	43	0.97	3.10	40	1.00	3.41	0.82	0.85	0.66
Total fungi (10^8)	98	3.86	3.59	100	4.53	2.92	100	4.78	4.47	0.76	0.32	0.18
Diversity[c], mean (SD)	100	6.22	2.37	100	6.61	2.49	100	7.04	3.40	0.26	0.24	**0.02**

Definition of abbreviations: n, number; LOD, limit of detection; CE, cell equivalents; GM, geometric mean; GSD, geometric standard deviation; SD, standard deviation.

[a]The percentage denotes the proportion of samples above the detection limit of the specific microbe or microbial components (total number: (1) endotoxin and glucan assays (n/n, respectively): the random sample (196/150), atopics (107/100), non-atopics (99/-); (2) microorganisms: the random sample (n = 192), atopics (n = 98), non-atopics (n = 96). Samples below the LOD (limit of detection) were assigned half of the LOD value (endotoxin (4.5 EU/m^2); β-(1,3)-D-glucan (0.18 µg/m^2); LOD for microorganisms, please see table S1). The β-(1,3)-D-glucan assay was not performed in non-atopics.

[b]t-test for differences in GM between microbial exposures in the different study groups. A p-value ≤0.05 in bold denotes a statistical significant difference between the atopis and non-atopics.

[c]Diversity denotes the number of different microorganism groups detected (range; 3–14).

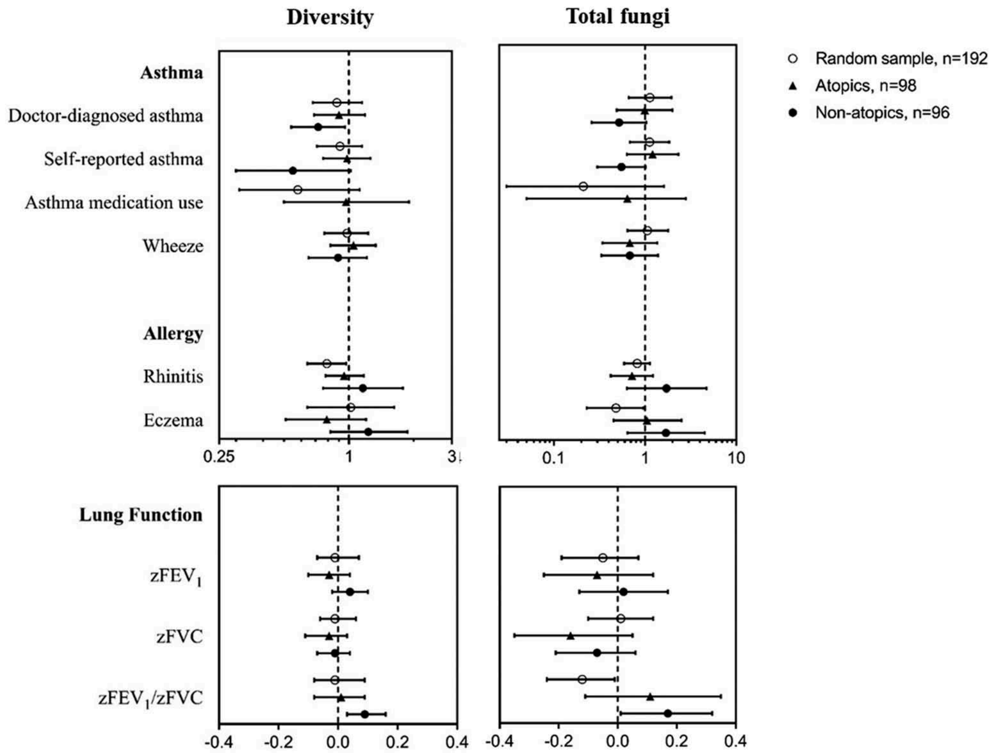

Figure 3. Associations of microbial diversity and total fungi with allergy, asthma and lung function in the random sample, atopics and non-atopics.

pooled all participants and performed analyses on microorganisms (individual species or groups of microorganisms, the total fungal load and diversity) with health outcomes while adjusting for atopy showing similar overall inconsistency in associations (data not shown).

Discussion and conclusion

To our knowledge, this is the first study to examine diverse domestic microbial exposures based on microbial DNA analysis of household dust and the associated allergic and respiratory health in adults. Self-reported household moisture damage was positively associated with asthma outcomes, and mould odor with wheezing in atopics. Inconsistent associations were found for individual microbial exposures and health outcomes, but we observed inverse associations between some microorganisms and doctor-diagnosed- and self-reported asthma in the non-atopics. Furthermore, our findings suggest that higher microbial diversity may be associated with less rhinitis and asthma, although this reached statistically significant for only some of the observed associations.

Among the other findings in our study, higher loads of β-(1,3)-D-glucan and endotoxin were associated with poorer health outcomes. This has previously been observed with β-(1,3)-D-glucan and airway obstruction [29] and airway inflammation in adults [29,30], as well as respiratory symptoms and peak expiratory flow variability in children [31–34]. Endotoxin induces airway inflammation and can cause and exacerbate respiratory symptoms in high doses. However, at lower doses, the epidemiological findings on respiratory symptoms, asthma and allergy are conflicting as positive, as well as negative and no associations have been reported in both children and adults [5,6,35,36]. Despite these inconsistencies, the epidemiological evidence indicates that in general, increased endotoxin levels may be associated with a lower risk of atopic sensitization, hay fever and atopic asthma, but a higher risk of respiratory symptoms [37–40].

In accordance with our findings in adults, only few significant associations have previously been reported between individual microbes and respiratory health in children [9,12,41]. A similar inverse association was observed between higher amounts of *Aspergillus fumigatus/Neosartorya fischeri* spp., *Cladosporium* spp. and having less asthma and/or wheeze in a study that included additional microbial spp. compared to our study [41]. In contrast to our findings, a positive association has been reported between *Streptomyces* DNA and doctor-diagnosed asthma [9] and an inverse association with FEV_1 [12]. Moreover, a case–control study found that *Aspergillus versicolor* DNA levels were slightly elevated in homes of allergic persons compared with homes of non-allergic persons' homes [14]. Also in homes of atopic children with mild and severe asthma, fungal exposure differed significantly in one study [42]. Due to the currently limited and inconsistent findings on the health effects of microbial exposure measured by microbial DNA, further investigation preferably based on a prospective design with repeated measurements is required in order to validate the current findings.

When comparing the present study to previous studies using similar methods for dust sampling and analysis, we found lower endotoxin loads [22,43,44], and lower β-(1,3)-D-glucan loads [19,45]. The reason for this is unknown but is likely to be attributed to methodological differences between studies [46]. Furthermore, in regards to our exposure assessment, the diversity score should be considered as a crude estimate of the overall microbial diversity and essentially only show fungal diversity as only one bacterial species (*Streptomyces spp.*) was included. Our findings indicate that increased microbial diversity may be associated with reduced allergy and respiratory outcomes in adults. A protective association of microbial diversity has also been shown previously with asthma [18,42] and wheeze [41] in children. Similarly, another study in asthmatic children showed that fungal concentration and composition was associated with increased risk of severe asthma, but in contrast low bacterial richness was associated with reduced risk of severe asthma [47]. Our findings indicate that the protective effect of a diverse microbial environment may also apply to non-farming environments, and to adults, rather than children. However, no conclusions on the potential mechanisms underlying this protective effect can be derived from the current cross-sectional study. The design allows us to establish only associations rather than causal relationships. Furthermore, we have only sampled dust once at single point in time. Health effects depend on prior, not subsequent or posterior exposures and therefore, we may not have measured exposures at the time most relevant for the investigated health outcomes. Additionally, we were unable to fully elucidate the association of total fungal load and specific microbial exposures due to the correlations between species. Nonetheless, our findings indicate that microbial diversity may be associated with allergy and respiratory health after adjustment for essential confounders. Further longitudinal investigation of indoor environmental dynamics and the associations between the indoor microbial flora and occupants' respiratory health are required to better identify the specific causal agents. An additional limitation of this study is that we performed multiple testing, increasing the risk of false-positive associations. Therefore, as this is a relatively small study with a large number of test performed, the observed associations should be considered as suggestive.

In contrast to the associations observed between moisture damage and asthma and mould and wheeze in atopics, no such associations were found in the non-atopics. However, due to the low prevalence of dampness problems in non-atopics, we were unable to evaluate whether atopics may be more susceptible to household dampness than non-atopics. Moreover, we cannot rule out that the observed associations in the atopics are occurring due to reporting bias as people reporting adverse health outcomes may be more likely to report dampness. The use of observations made by the investigators rather than the participants would be preferable. Moreover, the use of a prospective design is more desirable for temporal separation of exposure and outcome assessment. This design was applied in the European Community Respiratory Health Survey (ECRHS) comprising 7,104 adults from 13 countries, that showed an increased risk of new-onset asthma with water damage (RR, 1.46; 95% CI, 1.09 to 1.94) and with mould odor (RR, 1.30; 95% CI, 1.00 to 1.68) reported at baseline [1]. Additionally, in the ECHRS study, a stronger health effect of household dampness was observed in atopics compared with non-atopics [1], similar to our findings of an effect in atopics.

A strength of our study is the inclusion of lung function, which is a more objective measure than self-reported symptoms. However, we found no decline in lung function associated with dampness despite the fact that it has been observed in other studies [8,48,49] and no clear or consistent associations were found between species and groups of microorganisms and lung function. Furthermore, we were unable to identify potential microbial agents responsible for the observed association between dampness indicators and asthma, since we found only a few significant associations between individual microbial exposures and dampness indicators. It should, however, be noted that due to the low prevalence of some dampness indicators, we were underpowered to show any difference in levels of microbial exposure.

In conclusion, household moisture damage was associated with increased risk of asthma and mould odor with an increased risk of wheeze in atopics. Moreover, our findings suggest that asthma and allergy in adults may, in some cases, be associated with species composition and microbial load present in urban domestic environments.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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